Aberrant Crypt Foci and Microadenoma As Markers for Colon Cancer

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Foci of aberrant crypts similar to those seen in experimental animals exposed to colon carcinogens have been identified and quantified on the mucosal surface of fixed resections of human colon after methylene blue staining. Many of the foci in humans showed dysplasia on histologic examination and were considered to be microadenoma (MA). These lesions may be precursors for adenomatous polyps and colorectal cancer. Rats and mice initiated with azoxymethane, then fed diets containing sucrose or casein heated at 180°C to simulate normal cooking conditions, had three to five times more large MA after 100 days than controls. Thus, cooked sugar and protein contain promoters of the growth of colonic MA. 5-Hydroxymethylfuraldehyde was identified as a promoter in cooked sugar.

Colon cancer is thought to develop by a multistep process in which normal crypts are initiated to form foci of aberrant crypts (ACF) that proliferate by crypt fission to form microadenoma (MA). The MA enlarge to give macroscopic adenoma, adenomatous polyps, and finally adenocarcinoma.

Colonic cryps can easily be observed by examining the mucosal surface of the colon after it has been opened, fixed, and stained with methylene blue. When examined in this way, colons of animals treated with colon carcinogens 2 weeks earlier were found to have occasional, abnormally large, darkly staining, and slightly raised "aberrant crypts" (*1*). With longer times between carcinogen administration and observation, these aberrant crypts were frequently found as foci, with two to many hundreds of aberrant crypts appearing together in a cluster. Subsequent studies have shown that *a*) the aberrant crypts are induced in mice and rats specifically by colon carcinogens, including those derived from pyrolyzed protein (*2*), *b*) many aberrant crypts on histological examination show a high level of dysplasia and are correctly called microadenoma [MA (*3,4*)], and *c*) as MA grow they are associated with macroscopic adenoma and eventually colon cancer (*5*). A typical MA induced in a rat by azoxymethane is illustrated in Figure 1.

Using the appearance and growth of colonic MA in rodents as an end point, we recently initiated a series of studies to determine which factors in the Western diet might be involved in the initiation and promotion of colon cancer. We began by asking whether heating food components to simulate cooking would produce compounds that are promoters of MA. In the first experiment (*3*), CF1 mice and Fischer 344 rats were initiated with azoxymethane by standard procedures. One week after initiation, the animals were randomly assigned to one of eight diets with identical ingredients but with the three components sucrose, casein, and beef tallow, either uncooked or cooked. Control animals were given diets with uncooked ingredients, while experimental animals were fed diets in which one, two, or three of the components were cooked in an oven at 180°C until golden brown (simulating normal cooking conditions) before they were added to the diets at a level of 20% by weight. After the animals had been 100 days on the diets, their colons were fixed, stained with methylene blue, and scored for MA. The mice and rats fed cooked sucrose or casein and beef tallow cooked together had three to five times more large MA (defined so that there was an average of one large MA per control animal) than the controls (p, 0.02–0.0001). No significant increase in the number of MA was observed with the five other cooked diets. Two rats fed casein and beef tallow cooked together had adenocarcinomas.

In more recent work, we showed that beef tallow did not react chemically with casein but facilitated heat transfer to the protein. Thus, when casein is heated as a thin layer, it has the same activity as the casein–beef tallow mix. We may conclude, therefore, that thermolyzed sucrose or

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casein promote the growth of colonic MA in initiated mice and rats and would appear to contain promoters of colon cancer. In the case of sucrose, we have been able to show that the compound 5-hydroxymethylfuraldehyde (HMF) occurs in about 1% yield during thermolysis. HMF added to a control diet at a level similar to that present in the diet containing cooked sucrose produced an increase of the same magnitude as we observed for the cooked sucrose in the number of large MA in initiated rats. Furthermore, when HMF was removed from the cooked sucrose by solvent extraction, this material lost its ability to promote the growth of MA. It appears, therefore, that HMF is a promoter of the growth of colonic MA, and experiments are in progress to determine whether it promotes colon cancer. The identity of the promoting agent(s) in thermolyzed casein is also under investigation.

We have identified and quantified ACF and MA similar to those observed in the colons of experimental animals in both formalin-fixed and unfixed human colons stained with methylene blue (*6*). The average number of ACF/cm^2 in familial polyposis patients (20 ± 19, mean ± SD) was significantly higher than in patients with colorectal cancer

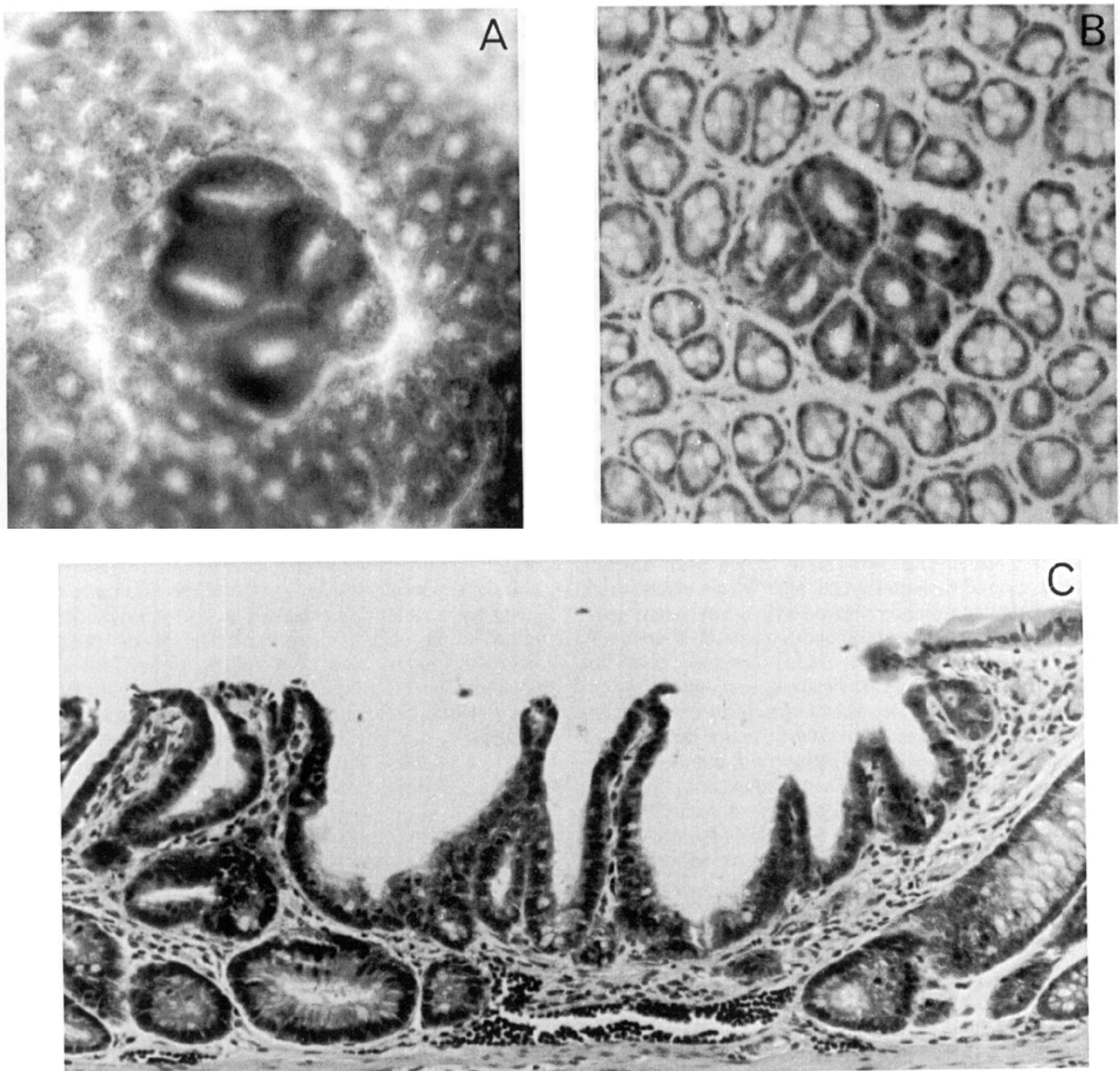

FIGURE 1. Aberrant crypt focus with high level of dysplasia (microadenoma; MA) from a rat initiated with azoxymethane. (*A*) Unsectioned mucosal surface stained with methylene blue (120 ×); (*B*) histology of section of MA cut parallel to mucosal surface showing high-grade dysplasia (H&E stain; 120 ×); (*C*) histology of section MA cut perpendicular to mucosal surface (H&E stain; 240 ×).

(0.37 ± 0.41) or benign bowel diseases (0.18 ± 0.35). At least 1 ACF was found in every colon from the cancer patients and in 6 out of 10 from the group with benign disease. The average number of crypts/ACF ranged from 5 to 35, with absolute values from 1 to over 100. In order to determine whether the topographic features of the ACF give an indication of the histologic appearance, 68 specimens containing ACF on normal mucosa were examined histologically (*4*). The presence of slitlike lumina in the crypts of ACF on the mucosal surface correlated with the presence of dysplasia at histology, thus identifying MA that may well be precursors for adenomatous polyps and colorectal cancer.

This parallel method for examining MA in experimental animals and man should make it possible to determine whether dietary factors that initiate and promote these putative precursor lesions in experimental animals have the same effects in humans.

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